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SHORT COMMUNICATION

THE OCCURRENCE OF MUTATOCHROME IN GREEN TISSUES

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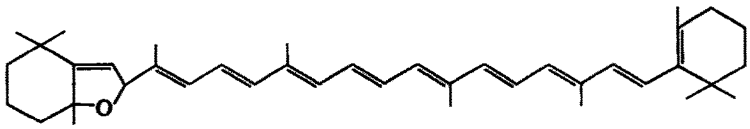
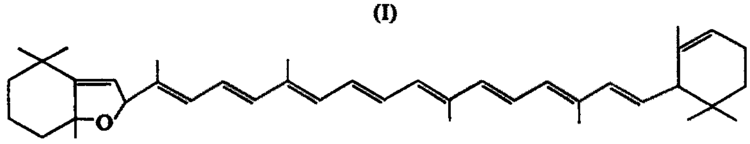
Abstract—Mutatochrome (5,8-epoxy-β-carotene) is present in green potato and maize leaves and in the alga *Fucus spiralis*. It was found along with what is probably flavochrome (5,8-epoxy-α-carotene) in carrot roots.

INTRODUCTION

IN EXPERIMENTS designed to detect the presence of β-zeacarotene and α-zeacarotene, possible precursors of β-carotene and α-carotene respectively, in green tissues, a pigment with the same absorption spectrum as β-zeacarotene but with different adsorptive powers was encountered in the leaves of potato, French bean and maize plants. It was finally identified as mutatochrome (5,8-epoxy-β-carotene) (I), a pigment first isolated as citroxanthin from orange peel.[1,2] This pigment was also noted in carrot roots and in this case it was accompanied by a pigment, spectroscopically identical with α-zeacarotene but with stronger adsorptive power, which appeared to be flavochrome (5,8-epoxy-α-carotene) (II).



(I)



(II)

RESULTS AND DISCUSSION

The unsaponifiable material (100 mg) from potato leaves was chromatographed on a column of alumina (Brockmann, grade III, 10 g). The carotenes were eluted with light petroleum (b.p. 60–80°; P.E.), and the orange zone containing the β-zeacaratene-like pigment with P.E. containing 2% ethyl ether. This zone was purified by rechromatography on a MgO:celite (1:1) column with 8% acetone in P.E. as developer. Its absorption maxima in

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[1] P. KARRER and E. JUCKER, *Helv. Chim. Acta* **27**, 1695 (1944).

[2] P. KARRER and E. JUCKER, *Helv. Chim. Acta* **30**, 536 (1947).

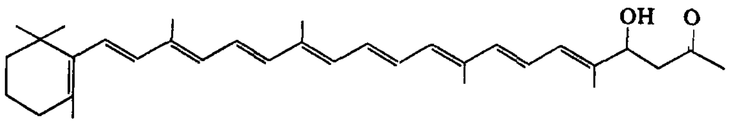
two solvents compared with β-zeacarotene are given in Table 1. Evidence that this pigment was not β-zeacarotene came from TLC on Kieselgel G with two solvent systems. The possibility that the new pigment was a more strongly adsorbed isomer of β-zeacarotene was also ruled out (Table 1). Treatment with I_2 did not affect its adsorptive power.

TABLE 1. COMPARISON OF ISOLATED AND KNOWN CAROTENES

	Adsorption maxima (mn)		Chromatography system	
	Solvent		Kieselgel G. with	
Compound	P.E.	Ethanol	7% benzene in P.E.	14% benzene in P.E.
β-Zeacarotene	404·5, 427, 452	405, 429, 454	0·70	0·88
Mutatochrome* (*a*)	404, 426, 452	405, 426, 453	0·00	0·06
(*b*)	— — —	405, 427, 454		
Mutatochrome after I_2 isomerization	—	—	0·00	0·06
"Flavochrome"	376, 399, 422, 449	—	0·05	0·13

(*a*) Ex carrots; (*b*) ex potato leaves.
* Davies.[3]

When the pigment was compared with an authentic sample of mutatochrome chemically synthesized from β-carotene, it had the same spectra in ethanol and P.E. and was not separated from the authentic sample on two thin-layer systems: (1) Kieselgel G with 80% benzene in P.E. as solvent, R_f 0·40; (2) alumina with the same solvent, R_f 0·45. It is interesting that another pigment more polar than β-zeacarotene, but with a very similar spectrum, exists in Nature; Yokoyama and White[4] isolated 8′-hydroxy-7′,8′-dihydrocitranaxanthin (III) from



(III)

the flavedo of Sinton citrangequat. The possibility that the pigment we isolated was this compound was ruled out by the action of HCl/ethanol; no effect was observed on our pigment or mutatochrome; with the citrus pigment there is a marked shift of the spectrum to higher wavelengths.[4] Furthermore, mutatochrome and our pigment were epiphasic in the phase test (P.E. and 90% aqueous methanol) and 7′-hydroxy-7′,8′-dihydrocitranaxanthin is hypophasic;[4] finally mutatochrome (citroxanthin) is also found in the epiphasic pigments of the flavedo.[4]

The same results were observed with extracts from maize and bean leaves (*Phaseolus vulgaris*) and from the alga *Fucus spiralis*.

In carrot extracts, however, the mutatochrome fraction separated into two zones when chromatographed on a MgO:celite (1:1) column. The more weakly adsorbed pigment, which was eluted with 6% acetone in P.E. had the same absorption spectrum as α-zeacarotene

[3] B. H. DAVIES, In *Chemistry and Biochemistry of Plant Pigment* (Edited by T. W. GOODWIN). Academic Press, New York (1965).
[4] H. YOKOYAMA and M. J. WHITE, *Phytochem.* **5**, 1159 (1966).

(376, 399, 422, 449 nm in P.E.) but was much more strongly adsorbed. As just indicated it was slightly less strongly adsorbed than mutatochrome (e.g. R_f 0·50 and 0·58 on kieselgel and alumina plates respectively, with 80% benzene in P.E. as developer in each case; see also Table 1) and would appear to be flavochrome, the α-carotene analogue of mutatochrome, which has been reported in flower petals (see Goodwin[5]). It was not possible to compare it with an authentic specimen.

EXPERIMENTAL

Extraction, separation and purification of pigments. Standard methods were used.[6, 7]

Materials. Potato leaves were Majestic. Maize was South African, horse tooth; carrots were G.P. Grand Prize—origin Texas; beans were Dwarf French bean var. Lighting. *Fucus spiralis* was collected on the shore at Aberystwyth.

Preparation of mutatochrome. β-Carotene (100 mg) was dissolved in chloroform (14 ml) and a solution of freshly prepared perbenzoic acid (30 mg in 1·5 ml chloroform) saturated with hydrogen chloride added. The mixture was allowed to stand in the dark for 3 days at 4°, and the chloroform solution then washed with water and sodium biocarbonate solution to remove benzoic acid, and finally several times with water to remove the bicarbonate. The chloroform solution was taken to dryness on a rotary evaporator and the residue chromatographed on alumina (Brockmann, Grade III). The unreacted β-carotene was eluted with P.E. and the mutatochrome with P.E. containing 1·5% ethyl ether.

Acknowledgements—R. J. H. W. was the holder of a Thomas and Elizabeth Williams Scholarship. This work was supported by a grant from the Science Research Council.

[5] T. W. Goodwin, *Comparative Biochemistry of the Carotenoids.* Chapman and Hall, London (1952).
[6] T. W. Goodwin, In *Modern Methods of Plant Analysis* (Edited by K. Paech and M. V. Tracey) Vol. 3, p. 372. Springer, Berlin (1955).
[7] R. J. H. Williams, G. Britton, J. M. Charlton and T. W. Goodwin, *Biochem. J.* In press (1967).